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Award Number: W81XWH-05-2-0081

TITLE: Military Nutrition Research: Four Tasks to Address Personnel Readiness and Warfighter Performance

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REPORT DATE: March 2006

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE
01-03-2006

2. REPORT TYPE
Final

3. DATES COVERED
1 Sep 2005 – 28 Feb 2006

4. TITLE AND SUBTITLE

Military Nutrition Research: Four Tasks to Address Personnel Readiness and Warfighter Performance

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-05-2-0081

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Donna Ryan, M.D.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Pennington Biomedical Research Center
Baton Rouge, LA 70808

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT: SEE NEXT PAGE

15. SUBJECT TERMS
nutrient intake, metabolism, nutrition, energy expenditure, readiness, performance

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	30	**19b. TELEPHONE NUMBER** *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

ABSTRACT

Background: Pennington Biomedical Research Center (PBRC) continues a 16 year collaborative effort with the Department of Defense (DoD) in this research effort. Objectives: To assess and evaluate novel ways to sustain warfighter performance during high intensity missions at home and abroad, under specially funded cooperative agreements between the US Army Medical Research and Materiel Command (USAMRMC) and PBRC, PBRC provides high quality analytical laboratory, nutrition database and metabolic unit support for military nical research protocols. . nutrition cliSpecific Aims: PBRC performs four research tasks as follows: Task 1: Clinical Laboratory for Human Samples; Task 2: Stable Isotope Laboratory; Task 3: Nutrient Database Laboratory; Task 4: Metabolic Unit Services. The four tasks supported 6 projects directed by USARIEM investigators. (1)The effect of fitness level, caloric intake, and protein intake on short-term nitrogen balance during a 1000-calorie increase in daily energy expenditure. (2)Nutritional Evaluation of the Meal, Cold Weather (MCW) as a Sole Source of Subsistence During an Extended Cold-Weather Operation (3)Effects of Caloric Deprivation and Meal Composition on Cognitive Performance and Glucose Levels (4) Effect of Tyrosine Supplementation on Cognitive Performance and Mood During Severe Cold Stress (5)Comparison of a PDA-based Method vs. Written Records for Assessing Energy Intake and Expenditure (6)Response of Biomarkers of Bone Remodeling to Military Recruit Training Study Design: In consultation with the Project Officer at US Army Research Institute of Environmental Medicine (USARIEM), PBRC Task Leaders for Tasks 1 and 2 determined the number, timing, type of sample and type of analysis. Analysis of relevant endpoints in the PBRC Clinical Laboratory and Stable Isotope Laboratory provided information useful for determination of energy expenditure, water turnover, body composition, clinical biochemistry and metabolism. In consultation with the Project Officer, the Task Leader for Task 3 was prepared to provide assistance with food intake analysis and nutrient composition. In consultation with the Project Leader, the Task Leader for Task 4 was prepared to provide access to the PBRC Metabolic Unit, where clinical studies of relevance to energy metabolism using state of the art facilities for metabolic chambers. Magnetic resonance spectroscopy is being installed at the Pennington Center and is scheduled for completion in June, 2006.

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INTRODUCTION

The PBRC has a 16-year history of collaborative research with the Department of Defense (DOD). A series of specially funded cooperative agreements between the PBRC and the U.S. Army Medical Research and Materiel Command (USAMRMC) has provided high quality analytical laboratory, nutrition database, and metabolic unit support for DOD nutrition related research programs. The program currently supports the RDT&E funded Military Nutrition Research Programs at the U.S. Army Soldier Systems Center (Natick, Massachusetts) and the U.S. Army Research Institute of Environmental Medicine (USARIEM) laboratories, as well as the Ration Sustainment Testing program. PBRC personnel have traveled to DOD field studies to collect samples, which are returned to the PBRC for laboratory analyses. Additionally, the PBRC has conducted research that complements and extends USARIEM's intramural program in areas of nutritional neuroscience, stress, physical, and mental performance, and garrison feeding. Though funded through earmarks, the PBRC program has been periodically successfully peer reviewed by an external panel from the Committee on Military Nutrition Research (CMNR), Institute of Medicine (1988, 1990, 1996, and 2002). This joint effort of PBRC and military researchers has led to significant improvements of operational rations, better understanding of warfighter energy and nutritional requirements, and modifications in garrison feeding.

This project continues a research program that has been in place since 1988. The project is for the fourth specially funded cooperative agreement series that began in 1988. The previous cooperative agreements are listed below.

Dates of Award	Title	Funding
7/1/97-12/31/04	Military Nutrition Research: Eight Tasks to Address Medical Factors Limiting Soldier Effectiveness	$16,710,748
4/1/92-3/30/98	Military Nutrition Research: Six Tasks to Address Medical Factors Limiting Soldier Effectiveness	$11,340,567
8/1/88-7/31/92	Effect of Food, Diet and Nutrition on Military Readiness and Preparedness of Army Personnel and Dependents in a Peacetime Environment	$3,845,000

In each of the three previous cooperative agreements, the CMNR of the National Academy of Sciences provided peer review prior to and during program implementation. Additionally, USARIEM approved all projects and provided consultation on research design for all projects that emanated from PBRC. Any modifications to the original research plan were approved by USARIEM prior to implementation.

The scope of work conducted by PBRC during the period covered by this annual report:

Task 1: Clinical Laboratory for Human Samples

- After consultation with U. S. Army Research Institute of Environmental Medicine (USARIEM), samples from USARIEM research projects were received, processed, and analyzed by the PBRC laboratory.
- The number, timing, type of sample and analyses performed were determined in consultation with USARIEM investigators.
- Results were forwarded to USARIEM and publications generated.

Task 2: Stable Isotope Laboratory

- After consultation with USARIEM, samples for analysis of energy expenditure, body composition and protein/metabolite turnover studies were analyzed by the PBRC laboratory.
- The number, timing, type of sample and analyses performed were determined in consultation with USARIEM investigators.
- Results were forwarded to USARIEM and scientific publications will be generated.

Task 3: Nutrient Database Laboratory

- After consultation with USARIEM, food intake analysis services can be performed and nutrient database services provided.
- The number and timing of field data collection services provided by PBRC personnel can be determined in collaboration with USARIEM.
- Results can be forwarded to USARIEM and scientific publications will be generated.

Task 4: Metabolic Unit Services

- Protocols can be developed in consultation with USARIEM and implemented using the PBRC metabolic unit clinical facility.
- PBRC can provide for metabolic kitchen services, inpatient unit nursing, clinical laboratory assessment, energy expenditure assessment, body composition assessment, food intake assessment and in-vivo bioimaging using multinuclear magnetic resonance spectroscopy.
- In consultation with USARIEM, scientific publications can be generated.

The four tasks were used to support the following six projects directed by USARIEM investigators:

1. Project 1 - The effect of fitness level, caloric intake, and protein intake on short-term nitrogen balance during a 1000-calorie increase in daily energy expenditure (current) (PI: CPT Matthew Pikosky, Ph.D.) - supported by Tasks 1 and 2.
2. Project 2 – Nutritional Evaluation of the Meal, Cold Weather (MCW) as a Sole Source of Subsistence During an Extended Cold-Weather Operation (current) (PI: Carol Baker-Fulco, MS,RD) - supported by Tasks 1, 2 and 3.
3. Project 3 - The Effects of Caloric Deprivation and Meal Composition on Cognitive Performance and Glucose Levels (current) (PI: Harris Lieberman, Ph.D.) - supported by Task 1.
4. Project 4 - Effect of Tyrosine Supplementation on Cognitive Performance and Mood During Severe Cold Stress (current) (PI: Caroline Mahoney, Ph.D.) -

supported by Task 1.

5. Project 5 - Comparison of a PDA-based Method vs. Written Records for Assessing Energy Intake and Expenditure (current) (PI: Gaston Bathalon, Ph.D.) - supported by Task 2.
6. Project 6 - Response of Biomarkers of Bone Remodeling to Military Recruit Training (current) (PI: Rachel Evans, Ph.D.) - supported by Task 1.

Project Description:

Project 1 - The effect of fitness level, caloric intake, and protein intake on short-term nitrogen balance during a 1000-calorie increase in daily energy expenditure .

Background

During heavy sustained military operations, nutrition is a major factor that impacts the health and performance of warfighters. However, for this population, the importance of dietary protein for "optimal" nutrition is not resolved. The extent to which total daily energy expenditure, caloric intake, and fitness level affect the balance between whole-body protein synthesis and whole-body protein breakdown and, thus, dietary protein requirements, remain debated. Sedentary individuals who increase activity exhibit a short-term increase in nitrogen loss from the body, assumed to reflect increased dietary protein requirement, but whether physically fit individuals, such as warfighters, respond similarly is unknown. Additionally, while whole-body protein catabolism increases when energy intake does not meet energy expenditure, the degree to which an increase in dietary protein may preserve whole-body protein synthesis and reduce catabolism, during inadequate intake and increased energy expenditure is unknown.

The purpose of this study is two fold: 1) to determine if, during increased energy expenditure, physically fit individuals exhibit a smaller, larger, or equivalent increase in protein requirement as sedentary individuals and 2) to determine if, during periods of heavy physical activity and inadequate intake, an increase in protein intake will enhance conservation of whole-body protein content.

Hypotheses

1. In fit and sedentary individuals, daily nitrogen balance becomes negative when TDEE increases significantly, even when energy intake matches expenditure.
2. Due to metabolic adaptations enhancing whole body protein synthetic capacity and/or blunting proteolysis, fit individuals will preserve nitrogen balance better than sedentary individuals when TDEE increases significantly.
3. For fit individuals who significantly increase TDEE, but not energy intake, consuming a greater fraction of energy as dietary protein will improve nitrogen

balance by enhancing whole-body protein synthetic rate, blunting proteolysis, or both.

4. For fit individuals who significantly increase TDEE, but not energy intake, consuming a greater fraction of energy as dietary protein will sustain or enhance gluconeogenesis while blunting proteolysis, and this effect will diminish with time as negative energy balance continues.

Technical Objectives

1. Over 7 days, determine nitrogen balance and measure changes in rates of whole body protein turnover and gluconeogenesis, in sedentary and fit individuals who increase TDEE by 1000 calories while consuming an energy sufficient diet that provides 55% of total calories from carbohydrate and 0.9 g protein/kg body weight.
2. Over 7 days, determine nitrogen balance and measure changes in rates of whole body protein turnover and gluconeogenesis, in fit individuals expending an additional 1000 calories while consuming a hypocaloric diet (deficit of 1000 calories) that provides 55% of total calories from carbohydrate and 0.9 g protein/kg body weight.
3. Over 7 days, determine nitrogen balance and measure changes in rates of whole body protein turnover and gluconeogenesis, in fit individuals expending an additional 1000 calories while consuming a hypocaloric diet (deficit of 1000 calories) that provides 55% of total calories from carbohydrate and 1.8 g protein/kg body weight.
4. Monitor plasma concentrations of the following:
    a. Hormones related to energy metabolism (i.e. insulin, glucagon, catecholamines).
    b. Metabolically active substances that affect protein metabolism (i.e. growth hormone, cortisol, cytokines).
    c. Products of energy metabolism (i.e. urea nitrogen, free fatty acids, glycerol).
5. Use the respirometry chamber to confirm that TDEE is both predictable and reproducible when a detailed activity schedule is followed.

Military Significance

This research addresses USARIEM STP III.B. Task 1 (Fueling optimal performance) and Task 2 (Enhancing/defending the body's biological matrix). Protein synthesis maintains physiological function, including immune response, neurotransmission, muscle mass and, thereby, performance.

Controlled studies investigating whole-body protein turnover and dietary protein requirement of physically fit individuals abruptly increasing total daily energy expenditure (TDEE) have not been conducted. At high levels of energy expenditure it is unclear how much dietary protein is required to meet the body's demand for energy and protein synthesis, or how a caloric deficit may impact this requirement. In fact, the 1999 Institute of Medicine report recommends research to quantitate the effect of energy deficit on protein requirements (1). The results of this experiment will be used to evaluate the adequacy of current Military Dietary Reference Intake (MDRI) and Nutritional Standards for Operational Rations (NSOR) for protein and to make recommendations regarding the optimal protein content of operational rations and/or nutritional supplements. In addition, data from this study will be used to further develop, refine, and validate the Dynamic Nutrition Model being developed by The U.S. Army Soldier and Biological Chemical Command.

Project 2 – Nutritional Evaluation of the Meal, Cold Weather (MCW) as a Sole Source of Subsistence During an Extended Cold-Weather Operation.

Background

This study is in direct support of a United States Marine Corps request for a study to determine whether deleterious effects occur when Marines consume the Meal, Cold Weather (MCW) ration for 21 days or more. The MCW replaces the Ration, Cold Weather (RCW) as the operational ration for cold-weather feeding. Like the RCW, it is composed of freeze-dried and dehydrated items that will not freeze and is higher in food energy than a ration based on 3 Meal, Ready-to-Eat (MRE) meals. The RCW was specifically formulated to contain moderate levels of sodium and protein to reduce body water requirements. The MCW, however, is based on the Food Packet, Long Range Patrol (LRP) restricted calorie ration and is not formulated to lessen body water needs. The MCW is significantly higher in sodium and protein than the RCW. Sole subsistence on the MCW will require about 1–1½ liters per day more water than the RCW.

This study is designed to support or refute the establishment of a 21-day field feeding policy for sole subsistence on the MCW in a cold-weather environment. A field test will take place at Fort Wainwright, Alaska with up to 120 volunteers from a company of the 172nd Infantry Brigade. Measurements will include: biochemical markers of general health, hydration and nutritional status; energy expenditure and total body water via doubly labeled water (DLW) on a representative sub-group of volunteers; dual energy X-ray absorptiometry (DEXA) and circumference measures to assess changes in body composition during the field training exercise (FTX); and dietary intakes by food record. Questionnaires will periodically be administered to determine mood state, environmental symptoms, and ration acceptability. The recommendation for a field feeding policy for the MCW will primarily be based on magnitude of weight loss, with consideration of hydration status changes.

Hypotheses

1. Warfighters subsisting on the MCW do not consume adequate energy and nutrients to prevent unacceptable levels of body weight loss and maintain performance and hydration and nutritional status.
2. Prolonged subsistence on the MCW affects individual mood and environmental symptomotology.

Technical Objectives

1. Establish whether warfighters solely subsisting on the MCW consume adequate energy and nutrients to prevent unacceptable levels of body weight loss and maintain performance and hydration and nutritional status.
2. Determine if prolonged subsistence on the MCW affects individual mood and environmental symptomotology.
3. Evaluate the acceptability of individual MCW components during prolonged feeding.
4. Describe nutritional supplementation practices of non-Special Operations Forces combat troops.

Military Significance

Elements of the Army's Objective Force may have to rely on individual operational rations for periods ranging from several days to a month. The MCW is a newly reconfigured individual operational ration intended for cold weather feeding. This study is in direct support of a request from Headquarters, USMC for a study to determine whether deleterious effects occur when Marines consume the MCW ration for 21 days or more). The one field test of the MCW, which was a 5-day study, captured only acceptability ratings. In addition, the menus in current production are substantially different from those field tested. The nutritional adequacy of the MCW as a sole source of subsistence has not been established. Furthermore, the MCW is significantly higher in sodium and protein than the RCW it replaces and, therefore, significantly increases body water requirements. This project is directly related to the research goal of the Ration Sustainment Program to ascertain that all field and operational rations provide adequate nutrition for the warfighter for the specific time intervals for which uses of the rations are intended.

Project 3 - The Effects of Caloric Deprivation and Meal Composition on Cognitive Performance and Glucose Levels.

Background

In the course of intense military operations warfighters frequently are unable to eat regular meals, and sometimes must go for several days without consuming any food. This study will determine whether a two-day period of caloric deprivation impairs cognitive performance and mood state. If decrements are observed, their time course will be quantified, and the types of cognitive performance that are affected by caloric deprivation will be determined. The extent of decrements, if any, will be determined by the use of several cognitive tests and the Profile of Mood States (POMS) questionnaire. In addition, this study will determine if variations in macronutrient formulation/ composition that affect plasma glucose levels differ in their effects on cognitive function. This will be determined by providing two isocaloric diets in addition to the caloric deprivation condition. One diet will be all carbohydrate and one a mixture of carbohydrate and lipids. To examine the physiological basis of changes in cognitive state associated with caloric deprivation and the different feeding regimes, glucose levels will be continuously monitored using a U.S. FDA-approved, minimally invasive sampling technique. In addition, selected metabolic markers in the plasma will be periodically assessed, by performing blood draws, to study other nutritional and endocrine factors that could be associated with the experimental treatment conditions and might prove useful indicators of cognitive capability. Successful completion of this study will permit us to provide quantitative information to warfighters and their leaders as to the importance (or lack there of) of providing adequate nutrition in intense military operations to ensure cognitive function is not adversely affected. We will also be able to determine what types of food are best consumed to maintain the optimum level of plasma glucose. In addition, this study will provide information on potential biomarkers of cognitive function that may be used as surrogate markers of warfighter cognitive function in research and military field applications.

Hypotheses

1 Cognitive performance and mood will be adversely altered by two days of caloric deprivation;
2. Lowered glucose levels will be associated in a quantitative manner with impaired cognitive function;
3. Treatment with slow release carbohydrate-lipid foods, compared to treatment with isocaloric carbohydrate foods, will result in improved cognitive performance and mood state for a longer period of time.

Technical Objectives

1. Assess cognitive performance and mood after two days of caloric deprivation;
2. Measure glucose levels after two days of caloric deprivation
3. Measure cognitive performance and mood state after treatment with slow release carbohydrate-lipid food or treatment with isocaloric carbohydrate foods.

Military Significance

During intense military operations and combat, warfighters often do not have the opportunity to eat. In multiple field studies, conducted in various environments with many types of units, it has been repeatedly observed that warfighters do not consume sufficient energy to meet their energy requirements. The decrements can be as much as 4000 Kcal a day - much more than the decrement that will be induced in the volunteers in this study. For example, in a training exercise conducted by the 75th Ranger Regiment over a 72 h period, the Rangers consumed on average less than 1300 Kcal total energy resulting in approximately a 4000 Kcal/day energy deficit. They lost on average 8.4 lb of weight in that brief period. While much of the weight loss was water, a significant portion was from other body compartments reflecting the effects of catabolism induced by negative energy balance. That exercise was not even remotely as intense as a true combat operation yet cognitive performance was severely compromised.

Furthermore, multiple anecdotal reports from medical staff participating in recent operations such as Enduring Freedom and Iraqi Freedom indicate that Special Forces units engaged in ground combat operations suffer large weight loss over brief periods of time. This protocol will study the role of inadequate dietary energy intake as a determinate of cognitive decrements and explore the effects of two dietary interventions - feeding rapidly absorbed carbohydrate alone versus a slow release carbohydrate/lipid combination. This will permit us to provide quantitative guidance to warfighters and their leaders as to the importance (or lack there of) of meeting the warfighters daily dietary energy and/or macronutrient requirements in intense military operations to ensure cognitive function is not adversely affected. Furthermore, if glucose levels are predictive of cognitive function under these circumstances, it may be possible to incorporate emerging non-invasive glucose monitoring technologies into Warfighter Physiological Status Monitoring (WPSM) ensembles. This information will allow warfighters and their leaders to know when it is critical to eat to preserve cognitive function. Technologies to monitor sleep and inform warfighters when rest is essential to maintain cognitive performance are also under investigation for inclusion in WPSM systems.

This research study will provide information in support of the following MRMC research programs: 1) Task Area 3.B - Nutritionally Optimized Future Warrior Assault Rations; 2) Task Area 3.C - Cognitive Function and Workload Biomedical Assessment Methods; 3) Ration Sustainment Testing (RST); and 4) Warfighter Physiological Status Monitoring. In addition, it will support Project JSN 99-2, Interactive Smart Foods for Combat Rations/Components of the DoD Combat Feeding Directorate, NRDEC, Natick, MA.

Project 4 - Effect of Tyrosine Supplementation on Cognitive Performance and Mood during Severe Cold Stress

Background

The overall objective of the research is to determine if supplementation with tyrosine, a large amino acid, will mitigate the cognitive impairments and the adverse effects on mood and symptoms associated with severe cold stress. Military volunteers completed two 1 1/2 hour immersions per test session in cold water (approximately 18°C) to cause an increased catecholamine turnover in the brain. Tyrosine or placebo was administered in a specially developed high-energy bar eaten before each immersion trial. Administration of the tyrosine or placebo was within-subjects and double blind. Measures of cognitive performance, symptoms and mood (as well as salivary measures of cortisol and testosterone) were taken at five time points during each test session.

Hypotheses

1. Cold water stress causes adverse changes in symptoms, mood and cognitive performance.
2. Tyrosine supplementation as compared to placebo will result in fewer adverse changes in symptoms, mood, cognitive performance, and thermal responses.

Technical Objectives

1. Evaluate if immersion in cold water causes adverse changes in symptoms, mood, and cognitive performance
2. Determine if participants treated with supplemental tyrosine before immersion in cold-water experience fewer adverse changes in symptoms, mood, cognitive performance, and thermal responses than when they are treated with placebo.

Military Significance

Military maneuvers in cold weather environments are adversely affected by mood and cognitive impairment associated with cold stress. This project seems to develop remedies.

Project 5 – Comparison of a PDA-based Method vs. Written Records for Assessing Energy Intake and Expenditure

Background

Dietary intake studies in military dining facilities have generally relied upon a visual estimation method to collect dietary data to assess energy intake. However, this method is only suitable for group feeding and, thus, is not feasible for studies involving free-living individuals (1). Reports show that energy intakes calculated from self-recorded food records are, on average, underestimated 10 – 30% when compared with energy expenditure determined by DLW or indirect calorimetry (2-4). The discrepancies are due mostly to underreporting of food intake which can be the failure to record all foods consumed or the underestimation of portion sizes. Likewise, research comparing written, self-reported physical activity against DLW demonstrates that self reports overestimate energy expenditure by approximately 10% (5-7). Clearly, there is a need to improve the self-reporting of food intake and physical activity in order to determine the energy balance of individuals with greater accuracy and precision (8).

Successful weight management requires a careful balance between energy intake (food and beverage consumed) and energy expenditure (activities of daily living). Self-monitoring of food intake and physical activity has been identified as one of the most effective strategies for weight loss and maintenance (9-10). Self-monitoring at the beginning of a weight loss program assesses eating and exercise behavior and continues throughout the program to evaluate behavioral changes and compliance with program goals (11). Evidence indicates that individuals who self-monitor their food intake and physical activities are more successful at weight loss than those inconsistent with self-monitoring (12). Current findings support the IOM recommendations of keeping a 3-day food intake record and a physical activity diary (or using an activity monitor such as a pedometer) every 3 months to help maintain weight loss (13). Therefore, valid and accurate self-monitoring techniques are necessary to assess deviations in energy balance to assist overweight and overfat Soldiers in successful weight loss and weight management (14-15).

Objective assessment of energy expenditure can be measured by a variety of methods, with varying degrees of accuracy. Components of TEE include resting energy expenditure (REE), which typically encompasses 50% - 70% of TEE; the thermic effect of food (TEF), which accounts for another 10%; and the remainder is physical activity. Physical activity is the most variable component (16) and is comprised of activities of daily living (bathing, feeding, and grooming, for example), sports and leisure, and occupational activities. The amount of TEE accounted for by physical activity and thus, the error of misreporting energy expenditure is greater for active individuals than sedentary.

Doubly labeled water, a reference method for validation of energy intake and expenditure, is a widely used criterion for estimating TEE in a free-living population (17). In DLW, TEE is calculated from carbon dioxide production and an estimate of the respiratory ratio and has been extensively validated against gas exchange in small animals and humans (18-19). When free living individuals are weight-stable, energy intake will be equal to TEE. Therefore, DLW allows investigators to compare the accuracy of self-reported energy intake.

Objective assessment of energy intake can also be measured by a variety of methods with varying degrees of accuracy. Seven-day weighted food intake records are often used when evaluating other dietary assessment methods such as the 24-hour recall, food frequency record, or other novel methods (20). Written self-reported food intake records are a common method for collecting energy intake. The volunteer writes descriptive information of all foods and beverages consumed over a period of time, typically 3 – 7 consecutive days (recording food intake for more than 7 days has shown greater error in reporting bias) (21). While keeping a food record, individuals are instructed to accurately describe all foods and beverages consumed including the name of the food/beverage (brand name, if applicable), preparation method(s), recipes for mixed foods and beverages, and accurate portion sizes. Portion sizes are measured (with a scale or other household measures such as cups or tablespoons), estimated against food models or pictures, or estimated without the use of an aid. Ideally, recording is done at the time the food(s) and/or beverage(s) is/are consumed, thereby, increasing recording accuracy of the foods and beverages consumed. Individuals often view the written food record as time-consuming. To reduce the omission of forgotten foods and clarify entries, the food intake records are reviewed with respondents by trained study personnel.

Studies indicates that underreporters of food intake are likely to be female (22), older (23), or overweight (24-25). Additionally, income, education (26), social desirability (27), body image, and history of dieting or restrained eating (28) are likely to influence accuracy of reporting. The Three-Factor Eating Questionnaire (TFEQ) is internally consistent and valid and is suggested as the best tool for psychometrically measuring the degree of dietary restraint (29). Therefore, we will control for dietary restraint by using results from the TFEQ such as disinhibition, hunger, and dietary restraint. Additionally, we will match groups for gender, age, and body mass index.

Because of the quantity and diversity of information collected in food intake records, coding (data entry) and nutrient analysis can be time consuming. A hybrid method of the “checklist” form in which respondents check off consumed food items at each eating occasion may be easier than recording a complete description of the food, and the costs of data processing can be minimized (30). The checklist method may be most effective when assessing a limited set of nutrients, e.g., macronutrient intake (carbohydrates, fats, proteins, and alcohol). Another type of checklist is the close-ended method consisting of foods in different food groups in which the respondent indicates whether that food group has been consumed concurrent with actual intake (31). Portion size is recorded in an open-ended manner or in specific categories. The BalanceLog® program utilizes a checklist-type database and therefore can potentially improve recording accuracy while simultaneously reducing the recording burden on volunteers and investigators.

BalanceLog® is a computer software program that helps individuals create a personalized program to reach and maintain their weight goals. It provides a database of more than 4,000 foods and 300 exercises in which an individual can accurately monitor energy intake and expenditure. The sources of nutritional analysis for foods in the database include the U.S. Department of Agriculture (USDA) National Nutrient Databases, brand name label data, restaurant data and data obtained from manufacturers. The caloric expenditure for each exercise in the database are based on metabolic equivalents from the Compendium of Physical Activities (32), the American College of Sports Medicine guidelines (33) and manufacturer supplied data for exercise equipment.

Although BalanceLog® is used as a self-monitoring tool for individuals to monitor energy intake and expenditure, no validation studies assessing the accuracy of the PDA-based BalanceLog® program compared to energy balance calculated from TEE as measured by DLW have been performed (personal communication, Haugen, January 2005). Likewise, utilization of BalanceLog® for recording energy intake and expenditure has not been compared with data obtained from written food intake and physical activity records. Therefore, the PDA-based BalanceLog® program is a novel, cost-effective method to estimate energy intake and expenditure in a free-living environment. The BalanceLog® tool may provide researchers a less resource-intensive approach to estimate energy intake/expenditure as well as providing Soldiers with a self-monitoring tool for weight management that reduces recording burden.

Hypotheses

BalanceLog® software used in conjunction with a PDA will be as accurate as written records for assessing energy intake and energy expenditure in weight stable volunteers when compared to TEE as measured by DLW.

Technical Objectives

1. Compare the accuracy of reported energy intake of written 3-day and 7-day food records and PDA-based software (BalanceLog®) against energy intake calculated from TEE as measured by DLW in weight stable volunteers.

2. Compare the accuracy of reported energy expenditure between written 3-day and 7-day activity records and PDA-based software (BalanceLog®) against TEE as measured by DLW in weight stable volunteers.

Military Significance

The 2002 Survey of Health Related Behaviors Among Military Personnel suggests that the military Services need to address rising rates of overweight among those on active duty, defined as a body mass index (BMI) > 25.0 kg/m^2 (34). The epidemic of overweight and obesity in the U.S. affects the military in that it reduces the pool of recruits that are of normal weight and decreases the retention of new recruits due to failure to adequately manage their body weights (11). As a result, Dr. William Winkenwerder, Jr., Assistant Secretary of Defense for Health Affairs, has identified overweight as one of five key challenges facing military medicine (35). With guidance from Dr. Winkenwerder and the Surgeons General, the DoD Prevention, Safety, and Health Promotion Council targeted overweight as a key health promotion issue in 2004. Clearly, weight management is a DoD targeted health concern and as a result, U. S. Army Medical Research and Materiel Command (USAMRMC) established Task Area 3.X, Weight Management Strategies, to emphasize the importance of research designed to aid overweight Soldiers.

Military personnel must successfully manage their body weight and body fat over the course of their careers or face the possibility of early discharge for noncompliance with AR 600-9, The Army Weight Control Program (36). In fact, in fiscal year 2003, 3017 Service personnel across the DoD were discharged for failure to meet weight/body fat standards; of these, 2705 were soldiers (personal communication, Grissom, September 2004). Recent reports indicate that the BMI of Army recruits is increasing as is the incidence of overweight in active duty Soldiers (37). Indeed, 62.3% of male and 32.4% of female Service personnel across the Department of Defense (DoD) are overweight, as defined by BMI > 25.0 kg/m^2 (38). Similar overweight prevalence rates of 60.1% for male and 40.6% of female Soldiers assigned to Forts Leonard Wood, Jackson, and Bragg were observed (39). We reported that 11% of male and 22% of female active duty Soldiers exceeded their screening weight-for-height and body fat standard and, therefore, meeting criteria to be placed on the Army Weight Control Program (unpublished observations, Bathalon, November 2004).

This project focuses on methods that can be considered for use in future studies of overweight and overfat Soldiers. The Army Surgeon General has authorized the use of 'Weigh-to-Stay' as the baseline standard of care intervention for Soldiers placed on the Army Weight Control Program. This education and intervention-based program provides overweight Soldiers with various techniques to help with weight loss. One strategy for successful weight loss and maintenance as discussed in 'Weigh-to-Stay' is self-monitoring of food intake and physical activities to monitor energy balance. Therefore, the PDA-based BalanceLog® program is a potential self-monitoring tool that Soldiers might use to manage their weight. In fact, 27% of male Soldiers and 32% of female Soldiers on the Army Weight Control Program at Fort Bragg reported that use of a PDA would be of interest to them for weight management (unpublished observations, Bathalon, January 2005). The use of PDA technology could advance self-monitoring for military (as well as civilian) volunteers participating in weight loss/maintenance programs. Thus, the protocol supports efforts to assist military personnel with weight management, a key health promotion and military retention issue (40).

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Project 6 - Response of Biomarkers of Bone Remodeling to Military Recruit Training

Background

Stress fracture, an overuse injury to bone, is one of the most common and potentially debilitating overuse injuries seen in military recruits. Stress fractures develop when unaccustomed mechanical loading results in fatigue microdamage at sites of accelerated bone remodeling. There are reports of clinical and experimental observations of abundant remodeling and associated porosity in the vicinity of stress fractures. High rates of bone remodeling can be identified by measuring biochemical byproducts of bone resorption and formation, however the utility of using bone turnover and other biomarkers of bone remodeling to assess the bone remodeling response during short-term training regimens is not well studied.

Hypotheses

Bone turnover markers and other serum markers may be useful for monitoring the course of bone adaptation during a 16 week period of recruit training.

Technical Objectives

To determine the clinical utility of using bone turnover markers and other serum markers associated with bone remodeling as noninvasive, sensitive tools for monitoring the course of bone adaptation during a 16-week period of recruit training.

Military Significance

Relatively little information is available regarding the effects of either acute or chronic exercise on biomarkers of bone turnover during military training regimens. The utility of using markers of bone turnover to monitor the bone remodeling response in military recruits is greatly understudied. Additionally, it is unclear whether women, who sustain stress fracture at much higher rates than men, have a greater bone turnover response to exercise. The results from this study will allow us to identify serum markers of bone formation and resorption that are sensitive to short-term physical training regimens, and that have the potential to be used in future studies that will attempt to relate observed changes in bone quality with changes in the formation:resorption ratio, particularly with respect to onset of stress fracture. Additional analyses of the relationship between bone turnover and the hormonal, dietary, and inflammatory response may lead to dietary or other medical interventions that optimize bone health of military service members during strenuous physical training regimens.

We provide below the background information for each of the tasks that are funded in this award.

1. Background - Task 1: Clinical Laboratory for Human Samples

The clinical laboratory provides support for military nutrition research by providing the following services:

a. assistance with protocol development
b. sample collection and processing on-site or in a field setting
c. sample analysis
d. new method development
e. assistance with manuscript publication

The laboratory is accredited by the Health Care Financing Administration (HCFA) and the College of American Pathologists (CAP) and participates in the lipid standardization program of the Centers for Disease Control. Good Laboratory Practices guidelines are being followed in the laboratory. The Clinical Research Laboratory is staffed by licensed medical technologists, phlebotomists, and accessioners.

The laboratory is well-equipped for performing routine and specialized tests on clinical subjects. In 2005 over 250 different analytical procedures involving 625,000 tests were performed by the lab. The laboratory is comprised of 5 departments: chemistry, special chemistry, point of care testing, hematology, and urinalysis. Testing is performed on a variety of specimen types including blood, urine, sweat, saliva, and feces. The Facilities Description in the Appendix provides a complete list of tests that are available in the Clinical Laboratory.

2. Background - Task 2: Stable Isotope Laboratory

The research conducted by the Stable Isotope Laboratory is in the area of energy and water requirements, and changes in body water, of soldiers, often under harsh environmental conditions. The method used to determine energy requirements is the doubly labeled water (DLW) technique, which involves oral administration of water labeled with the stable isotopes, ^{2}H and ^{18}O. Saliva and urine samples are then obtained for periods of four to 14 days, longer with redosing. Water intake can be determined using only the ^{2}H labeled water. The use of doubly labeled water for measurement of energy expenditure was developed as a field technique for use in small animals (1)*. The method is based on the premise that after a loading dose of $^{2}H_2{}^{18}O$, ^{18}O is eliminated as CO_2 and water, while deuterium is eliminated from the body as water. The rate of CO_2 production, and, hence, energy expenditure, is calculated from the difference of the two elimination rates. The only requirement of subjects is to give urine and saliva specimens before and after drinking an initial dose of $^2H_2{}^{18}O$, and then return in one to two weeks to give a final urine specimen. During the period between the two urine and saliva samplings, subjects are free to carry out their normal activities and are not required to maintain extensive diaries. The doubly labeled water method has been extensively validated in humans under controlled settings (2), but there are confounding factors that need to be considered in field studies, particularly in Army Field Studies. Among these are change in location or food and water supply immediately preceding, or during an energy expenditure study. These changes may cause a change in baseline isotope abundance and, therefore, interfere with the accuracy of the energy expenditure measurement. This has occurred in a previous field training exercise involving the study of the MRE and RLW rations (3). This is a particular problem with studies such as the Ranger Training Studies (4), in which soldiers are moved to different parts of the country during the study. Therefore, a group not receiving labeled water must be followed to make any corrections in baseline isotope shifts.

Hydration status is another main focus for some Army studies. Using the cheaper and more readily available deuterium tracer, either changes in total body water (5,6) can be followed during a study, or water turnover (intake) (7,8) can be measured during a study.

One advantage of the DLW method is that it uses stable isotopes so there is no radiation exposure. The method uses two heavy isotopes of water, which are naturally occurring in food and water. There are no known side effects of either isotope at the doses given in DLW studies and has been used extensively to study energy expenditure during pregnancy (10,11) lactating women (12), and infants for measurement of energy expenditure and human milk intake (13-15).

In addition to the DLW studies, the PBRC Stable Isotope lab has provided analytical and technical support to examine protein turnover and gluconeogenesis using stable isotope tracer technology in a clinical protocol examining the effects of protein supplementation during caloric restriction (Project 1). The purpose of the study is two fold: 1) to determine if, during increased energy expenditure, physically fit individuals exhibit a smaller, larger, or equivalent increase in protein requirement as sedentary individuals and 2) to determine if, during periods of heavy physical activity and inadequate intake, an increase in protein intake will enhance conservation of whole-body protein content.

The lab determined the appropriate methods to quantitate glucose appearance, disappearance (6,6 d_2 glucose) and gluconeogenesis (2-^{13}C glycerol). The laboratory also decided on the appropriate tracers to quantitate protein synthesis (^{15}N phenylalanine, 2,3,5,6 D_4 tyrosine, and ^{15}N tyrosine).

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3. Background - Task 3: Nutrient Database Laboratory

Assessing dietary intake is essential in determining the soldier's nutritional needs and how those needs interface with other aspects of military performance. PBRC currently participates in field studies planned and conducted by the Military Nutrition Division of USARIEM by providing assistance with and analysis of dietary intakes collected during military field studies. That participation includes the following:

- Support for USARIEM field studies requiring data collection and data entry needs
- Support for PBRC in-house Military Nutrition Tasks
- Continued programming efforts directed toward meeting computer needs of both USARIEM and PBRC Military Nutrition Tasks

The Nutrient Database Integration Laboratory provides essential services for military operations. This Task oversees the operation of MiDAS, the database containing nutrient information for all operational rations, in addition to the USDA Standard Reference Foods and the USDA Food Survey Database food files. The Task provides critical support to studies which seek to improve soldier nutrition in a variety of field settings. The Laboratory is currently integrating all Armed Forces Recipes, special formulations (MREs) and other food formulations merged into one centralized database system at PBRC to be maintained and updated for USARIEM's use. This project is entitled "MRE Nutrient Composition: Integrating MREs into the Army Database Files" and the PI is Dr. Andrew Young.

The Task Leader and her staff are proficient in all aspects of nutritional intake assessment, including the Food Frequency Questionnaire, Food Diary Analysis, and Dietary Recall. The Leader and staff have been trained in the USDA multiple pass methodology. The Task Leader is a trained food scientist and has unsurpassed knowledge of nutrient database operations.

4. Background - Task 4: Metabolic Unit Services

In 1993 the PBRC Metabolic Unit was used for a special inpatient study. A description of this experience serves to illustrate how other studies might be developed to address military problems.

The PBRC served as the site for two research cohorts of U.S. Army Rangers. That project was, "Assessment of Intra- and Inter- Individual Metabolic Variation in Special Operations Forces Soldiers." The PI for the project was Ms. T. E. Jones, affiliated with the Military Nutrition Division at USARIEM. Co-Investigators were C. Gabaree, Lt. Col. T. C. Murphy, Donna Ryan, M.D., and E. Brooks, R.N., M.N.

The purpose of the study was to evaluate a group of Special Operations Forces (SOF) volunteers to determine the metabolic variation during rest, exercise and post-exercise recovery of the individual soldiers. On June 11, 1993, 10 SOF soldiers arrived to serve as the first cohort for testing. Army personnel at the PBRC included Tanya Jones (PI), Sven Ljamo, M.D. (Medical Monitor), Catherine Gabaree (Exercise Physiologist), Lt. Col. Cliff Murphy (Dietitian) and three civilian spotters for exercise testing. The first cohort of military volunteers and civilians left the PBRC on July 1, 1993. There were minimal complications that occurred in the SOF volunteers (subungual hematomas, muscle soreness, and poison ivy dermatitis). All procedures were carried out safely and satisfactorily. A mid-course correction session at the end of the first cohort stay resulted in minor procedure adjustments. From July 9-24, 1993, 10 members of the SOF from the 10th SFG at Fort Devens, Massachusetts participated in the study. All procedures were carried out safely and satisfactorily.

The Metabolic Unit project demonstrated that carbohydrate loading produced increments in physical performance in SOF soldiers. However, the variation between individual soldiers was not great enough to support developing individualized carbohydrate supplements. As a result of this work, the SOF did not pursue a plan to develop individualized soldier supplements for SOF. Therefore, this lack of metabolic variation does not mean that carbohydrate loading would not be effective and the military will pursue carbohydrate loadings for high intensity exercise operations for our SOF soldiers.

The PBRC Metabolic Unit was also used for studies on sleep deprivation (16,17). These included a comparison of tyrosine, against phentermine, caffeine, and d-amphetamine during sleep deprivation with analysis of effects on sleep and on cognitive and motor performance. PBRC has also been a site for a clinical study for the evaluation of minimal or non-invasive methods for field assessment of nutritional and metabolic status. Cpt. Mark Kellogg served as the PI for this project. We have been asked by USARIEM to continue this project's availability for the upcoming grant cycle and have plans to expand our facilities to magnetic resonance spectroscopy studies to accommodate projects in 2006 and beyond.

We are expanding our Metabolic Unit capabilities with a multinuclear magnetic resonance system, installed in this year and described in this report. The system operates at strength of 3.0 Tesla and sets a new standard for state-of-the-art in situ biochemistry and imaging. The system contains multiple expandable channels and high bandwidth receivers that ensure unparalleled image reconstructions from all pulse sequences. The multinuclear system allows for the tracing of hydrogen, carbon, and phosphorus atoms within biological molecules such as glucose and fatty acids.

This instrument is critical for the development of research protocols that will determine the optimal nutrients for military performance. Similarly, this instrument can be used to probe the basic biochemistry of the cell, evaluate ergonomic aids, and identify defects in cellular metabolism that are related to peak physical performance. This technology is not currently available within the DoD research environment and will provide state-of-the-art capabilities in military nutrition research. Peak physical performance is required for the elite warfighter. At its core, physical performance is the utilization of nutrients to perform work. Most of our knowledge of the energy producing mitochondria comes from animal studies or ex-vivo studies. This is obviously an artificial situation. The emerging field of magnetic resonance spectroscopy allows us to probe the biochemistry of the cell in vivo in a non-destructive manner, with no radiation and during the performance of work. These kinds of studies are important, because our understanding of how nutrients influence cellular bioenergetics in-vivo is weak.

BODY

Task 1 - Clinical Research Laboratory for Human Samples

<u>Key Research Accomplishments</u>

- Recertification by the College of American Pathologists.
- Continued excellence in the Centers for Disease Control lipid standardization program.
- Completion of blood, saliva, sweat, and fecal testing for Project 1. Specifically, the following tests were completed: glucose, creatinine, blood urea nitrogen, cortisol, growth hormone, insulin, free fatty acids, beta hydroxy butyrate, glucagon, and IGF-1, epinephrine, norepinephrine, urine creatinine, urine total nitrogen, urine urea, urine epinephrine, urine norepinephrine, sweat total nitrogen, and fecal total nitrogen. A total of 2604 assays were completed for this project.
- Completion of all testing for Project 2. Specifically, the following tests were completed: salivary caffeine, cortisol, and testosterone. A total of 618 assays were completed for this project.
- Completion of all testing for Project 3. Specifically, the following tests were completed: glucose, fructosamine, total protein, and albumin. A total of 2268 assays were completed for this project.
- Completion of all testing for Project 4. - Specifically, the following tests were completed: salivary cortisol and testosterone. A total of 466 assays were completed for this project.

- Completion of all testing for Project 6. Specifically the following tests were completed: albumin, bone specific alkaline phosphatase, calcium, CTP, IL-1B, IL-6, PINP, PTH, TNFa, TRAP, and vitamin D. A total of 6,270 assays were completed for this project.

Reportable Outcomes

Nindl B, Alemany H, Kellogg M, Rood J, Allison S, Young A, Montain S. Utility of insulin-like growth factor-I as a biomarker for assessing short-term metabolic stress in healthy men. *AJCN* (submitted), 2006.

Conclusions

The clinical research laboratory and food analysis laboratory continue to provide valuable support services to enhance nutrition research in the military. The laboratory plays an important role in furthering the knowledge concerning nutrition in the military by providing routine and esoteric testing, custom method development, assistance with testing and collection protocols, field assistance with blood collection and processing, and collaboration on protocols.

References

None.

Task 2 - Stable Isotope Laboratory

Key Research Accomplishments

- Completion of saccharic acid, glucose, and amino acid assays for 20 subjects. Work is continuing on the remaining 10 subjects.
- Completion of all assays for project 4. Total body water determination and water turnover studies were performed for 10 subjects.
- Completion of all assays for project 5. Total body water determination and water turnover studies were performed for 26 subjects.

Reportable Outcomes

None

Conclusions

The Stable Isotope Laboratory continues to provide valuable support services to enhance nutrition research in the military. The laboratory plays an important role in furthering the knowledge concerning nutrition in the military by providing research expertise and sample analysis in the area of energy and water requirements, and changes in body water. In addition to the labeled water techniques, the laboratory plays a key role in furthering knowledge on protein turnover and gluconeogenesis using stable isotope tracer technology.

References

None.

Task 3 - Nutrient Database Laboratory

Key Research Accomplishments

- Continued development of MENu and MiDAS applications for Military Nutrition use.
- Further development of digital photography methodology for use in dietary assessment of soldiers' intakes.

Reportable Outcomes

Although there are no currently active field studies that we are supporting we continue to update and enhance our database capabilities to support future studies. Our services include participation in the field and utilization of the internet as a more efficient means of access to the MENu and MiDAS databases. We are also working to further incorporate the digital photography methodology for dietary assessment of soldiers' intake for use when relevant studies are in the planning stages. Modification of this methodology and work on internet application of the databases and software programs developed for military nutrition efforts has continued during this year.

Conclusions

We are working to expedite efforts on our end to incorporate state of the art technology for use in field trials in the Military Nutrition Division of USARIEM. We anticipate that additional modifications will occur during the next year to develop a system that can meet the needs of field experiments in a timely manner. We will be ready to support future studies with very little lead time.

References

None.

Task 4 - Metabolic Unit Services

Key Research Accomplishments

- Installation and initial calibration and operation of a 3T MRI / MRS.
- Developed policy, procedure and completed training for MR safety.
- Initiated collaborations for the development of ^{1}H intramyocellular and intrahepatic lipid measurement and for ^{31}P ATP synthesis rates by phosphocreatine turnover.
- Acquired first images of body composition and first ^{1}H intramyocellular lipid spectra.

Reportable Outcomes

Although there are no currently active metabolic unit studies that we are supporting we continue to update and enhance our capabilities to support future studies through the development of MRI /MRS. We are also working to further enhance our capabilities in skeletal muscle mitochondrial function through the finalizing and validating the ^{31}P ATP synthesis rate measurement and plan to incorporate near-infrared combined oxy/deoxyHb oxy/deoxyMb measurements of oxygen consumption in order to directly measure P/O ratios.

Conclusions

We anticipate that additional development of the MRS system over the next year and becoming fully operational for mitochondrial measurements in about six months.

References

None.